SODIUM TRIPOLYPHOSPHATE

TOXICITY and TERATOGENICITY STUDIES
in Avian Embryos

A31

GENERAL PROCEDURES

The protocols as specified under FDA Contract #72-345 were followed in the investigation of toxicity and potential teratogenicity of the specified substance. The toxicity of the substance was evaluated from the percentage hatch of embryos injected either in the air cell or yolk at either zero hours (pre-incubation) or after 96 hours incubation to provide four separate evaluations.

EGG SOURCE AND HANDLING

All eggs used in these investigations were from Shaver Starcross pullets housed at the Poultry Research Center of the University of Arizona in Tucson. The parent stock was maintained on the University of Arizona breeder diet which had been formulated to provide more than adequate amounts of all the known nutrients required by the breeding hen.

The feed was specially prepared to assure no contaminations and did not contain any additive drugs such as antibiotics. All eggs prior to use (within 48 hours of lay) were candled to remove any containing blood spots, abnormal air cells or abnormal shells, and only clean eggs ranging in weight from 23 - 26 ounces per dozen were used.

The supply flock was tested to assure the absence of Pullorum and Mycoplasma gallisepticum.

The eggs were incubated in forced draft Jamesway 252 machines with automatic temperature and humidity controls and an automatic turning device.

COMPOUND HANDLING FOR INJECTION

The substance tested was solubilized in a number of the prescribed solvents in order to determine the maximum concentrations which could be employed. Where possible, water was the solvent of choice. Maximum

injection volume was 0.05 ml. and all solvents and glassware were autoclaved prior to preparation of the solutions for use. The dose levels were administered with a microliter syringe using sterilized needles.

The preliminary range-finding studies using each of the administration routes and times were carried out with 10 - 25 eggs per dose level and included solvent controls, untreated controls and either drilled or pierced controls.

The actual dose-response protocol was carried out in two or more injections on different days to produce a minimum of 100 eggs at each dose level in five or more levels selected from the range- finding studies.

EXAMINATIONS OF EMBRYOS AND CHICKS

Eggs were candled daily and the dead embryos removed, examined and any abnormalities recorded. Five chicks from each dose level in each hatch were X-rayed to determine any skeletal abnormalities. Additional eggs injected at the approximate LD-50 level and an additional level below that were incubated and embryos at 8, 14, 17 days and hatch chicks removed for histopathological examinations.

In additional studies representative chicks from the dose-response protocol were saved. These chicks were housed in electrically-heated battery brooders with raised wire floors and fed University of Arizona diets. Feed consumption and growth rates were evaluated at 6 weeks of age and a sample of the birds sacrificed for gross and histopathological examinations.

DATA HANDLING

All data were coded on forms provided by FDA for computer input. In addition to summaries of mortalities and abnormalities, a number of statistical evaluations were carried out. These statistical analyses included the following for both mortality and the incidence of abnormal embryos:

1. Chi-square tests for all dose levels and for each level against the solvent control.
2. Linear regression analyses + chi square test of linearity.
   a. % response against dose
   b. % response against log dose
   c. log % response against dose
   d. arcsin transformation against dose
   e. arcsin transformation against log dose
3. Log dose against Probit using Finney's maximum likelihood method.
   a. Where significant, the LD-30, 50, 70 and 90's were estimated with 95% confidence intervals.
4. One-way analyses of variance.
5. Linear regression with replication.

Sodium tripolyphosphate (71-46) was solublized in water for use in the test protocols. The highest dose level of 125 mg/kg was obtained with a solution containing 125 mg/ml.

MORTALITY

Mortality data resulting from the studies using the four test protocols are shown in Tables 1 - 4. Chi-square analyses of the mortality data suggest that sodium tripolyphosphate was toxic to embryos under each of the four protocols. When air cell-0 hr administration was used doses of 5 mg/kg and above significantly increased embryo mortality in comparison with the solvent control groups. Chi-square analyses of the air cell-96 hr data indicate that 50 mg/kg and above were embryo toxic (Table 5). When the yolk administration route was employed for either 0 or 96 hour injection times, dose levels of 25 mg/kg and above elicited significant increases in mortality.

Probit analyses of these data resulted in a significant linear relationship ($P < 0.05$) for the air cell-96 hr series with an LD-50 estimate of 50.1 mg/kg. The yolk-0 hr series was significant at the 0.1 level of probability with an LD-50 estimate of 620.8 mg/kg (Table 6). The other two series did not yield a significant linear relationship between log dose and probit of mortality.

TERATOLOGY

The incidences of abnormal embryos and those showing H-S-V-L abnormalities are shown in Tables 1 - 4. The occurrence of abnormalities

in these series with the injection of sodium tripolyphosphate was quite low, and Chi-square analyses of these data failed to indicate a statistically significant difference between those groups treated with the test substance and those receiving water as a solvent control (Table 7). Probit analyses likewise failed to suggest a statistically significant linear relationship between log dose and probit of abnormality incidence in any of the four test protocols (Table 8).

Chi-square analyses of the data relating to H-S-V-L abnormalities were also not statistically significant at the 0.05 level of probability (Table 9). These data suggest that sodium tripolyphosphate was not teratogenic under the conditions employed in these studies. The individual teratogenic findings obtained in these studies are shown in Table 10.

TABLE 1

SODIUM TRIPOLYPHOSPHATE
in WATER
AIR CELL - 0 HRS

Dose, ppm	No. Fertile	Mortality %	Mortality #	Abnormal Total %	Abnormal Total #	Abnormal H-S-V-L %	Abnormal H-S-V-L #	Abnormalities by category: Head %	Head #	Skeletal %	Skeletal #	Viscera %	Viscera #	Limbs %	Limbs #	Structural %	Structural #	Toxic Response %	Toxic Response #	Functi %
125.0	166	29.51	49	1.20	2	1.20	2	0.60	1			0.60	1							
100.0	123	21.95	27	1.62	2	2.43	3	0.81	1			1.62	2							
50.0	124	20.16	25	0.80	1	0.80	1					0.80	1							
25.0	125	12.80	16	0.00	0	0.00	0													
5.0	127	22.83	29	1.57	2	1.57	2					1.57	2							
0.0	165	12.12	20	0.60	1	0.60	1	0.60	1											
drilled	125	11.20	14	0.80	1													0.80	1	
ntreated	387	11.62	45	1.03	4	1.03	4	0.77	3			0.25	1							0.25

SUMMARY - ALL DOSE LEVELS

665	21.95	146	1.05	7	1.20	8	0.30	2		0.90	6					

TABLE 2

SODIUM TRIPOLYPHOSPHATE
in WATER
AIR CELL - 96 HRS

Dose, ppm	No. Fertile	Mortality %	Mortality #	Abnormal Total %	Abnormal Total #	Abnormal H-S-V-L %	Abnormal H-S-V-L #	Abnormalities by category: Head %	Head #	Skeletal %	Skeletal #	Viscera %	Viscera #	Limbs %	Limbs #	Structural %	Structural #	Toxic Response %	Toxic Response #	Functiona %	Functiona #
125.0	119	98.31	117	0.00	0	0.00	0														
100.0	178	97.75	174	0.56	1	0.56	1					0.56	1			0.56	1				
50.0	177	57.06	101	2.25	4	2.25	4	0.56	1			1.12	2	0.56	1	1.12	2				
25.0	216	16.66	36	0.92	2	0.46	1							0.46	1	0.46	1				
10.0	58	6.89	4	0.00	0	0.00	0														
5.0	119	14.28	17	0.84	1	0.84	1	0.84	1												
2.0	59	10.16	6	0.00	0	0.00	0														
0.0	209	13.87	29	0.47	1	0.47	1					0.47	1								
rilled	118	6.77	8	0.84	1	0.00	0													0.84	
reated	387	11.62	45	1.03	4	1.03	4	0.77	3			0.25	1							0.25	

SUMMARY - ALL DOSE LEVELS

926	49.14	455	0.86	8	0.76	7	0.22	2		0.32	3	0.22	2	0.43	4				

TABLE 3

SODIUM TRIPOLYPHOSPHATE
in WATER
YOLK - 0 HRS

Dose, ppm	No. Fertile	Mortality %	Mortality #	Abnormal Total %	Abnormal Total #	Abnormal H-S-V-L %	Abnormal H-S-V-L #	Abnormalities by category: Head %	Head #	Skeletal %	Skeletal #	Viscera %	Viscera #	Limbs %	Limbs #	Structural %	Structural #	Toxic Response %	Toxic Response #	Functiona %	Functiona #
125.0	122	49.18	60	1.63	2	0.81	1	0.81	1											0.81	
100.0	126	60.31	76	0.79	1	0.00	0											0.79	1		
50.0	121	47.93	58	1.65	2	2.47	3	1.65	2			0.82	1								
25.0	122	48.36	59	2.45	3	2.45	3	1.63	2			0.81	1								
5.0	158	39.87	63	1.26	2	1.89	3	0.63	1			1.26	2								
0.0	163	25.76	42	3.06	5	0.61	1	0.61	1							1.22	2	0.61	1	0.61	
ierced	122	41.80	51	1.63	2	0.81	1					0.81	1			0.81	1				
reated	387	11.62	45	1.03	4	1.03	4	0.77	3			0.25	1							0.25	

SUMMARY - ALL DOSE LEVELS

649	48.69	316	1.54	10	1.54	10	0.92	6			0.62	4					0.15	1	0.15	1

TABLE 4

SODIUM TRIPOLYPHOSPHATE
in WATER
YOLK - 96 HRS

Dose, ppm	No. Fertile	Mortality %	Mortality #	Abnormal Total %	Abnormal Total #	Abnormal H-S-V-L %	Abnormal H-S-V-L #	Abnormalities by category: Head %	Head #	Skeletal %	Skeletal #	Viscera %	Viscera #	Limbs %	Limbs #	Structural %	Structural #	Toxic Response %	Toxic Response #	Functiona %	Functiona #
125.0	154	44.80	69	1.94	3	1.29	2	1.29	2									0.64	1		
100.0	119	30.25	36	3.36	4	1.68	2	0.84	1			0.84	1			1.68	2	1.68	2		
50.0	118	44.06	52	2.54	3	0.84	1	0.84	1							0.84	1	0.84	1		
25.0	117	33.33	39	5.12	6	1.70	2	0.85	1					0.85	1	1.70	2	0.85	1	0.85	1
5.0	118	19.49	23	0.84	1	0.00	0											0.84	1		
0.0	158	20.88	33	0.63	1	0.63	1							0.63	1						
erced	79	24.05	19	0.00	0	0.00	0														
eated	387	11.62	45	1.03	4	1.03	4	0.77	3			0.25	1							0.25	1

SUMMARY - ALL DOSE LEVELS

626	34.98	219	2.72	17	1.12	7	0.80	5		0.16	1	0.16	1	0.80	5	0.96	6	0.16	1

TABLE 5

SODIUM TRIPOLYPHOSPHATE
CHI-SQUARE ANALYSES of MORTALITY

Dose Level mg/kg	Air Cell		Yolk	
	0 hrs	96 hrs	0 hrs	96 hrs
2.000	-	0.278	-	-
5.000	5.156*	0.004	6.627	0.018
10.000	-	1.448	-	-
25.000	0.000	0.441	14.596*	4.764*
50.000	2.896	78.102*	14.003*	15.963*
100.000	4.292*	267.823*	33.699*	2.702
125.000	14.142*	215.510*	15.641*	19.205*
All Doses (DF)	20.783*(5)	612.057*(7)	39.710*(5)	37.123*(5)

* Probability < 0.05 - 0.005.

TABLE 6

SODIUM TRIPOLYPHOSPHATE
PROBIT ANALYSES - MORTALITY

	Air Cell		Yolk	
	0 hrs	96 hrs	0 hrs	96 hrs
		mg/kg	Sig. P<0.10 mg/kg	
LD-30	NS	40.8	31.5	NS
LD-50	NS	50.1	620.8	NS
LD-70	NS	61.6	12224.1	NS
LD-90	NS	82.9	903720.4	NS

TABLE 7

SODIUM TRIPOLYPHOSPHATE
CHI-SQUARE ANALYSES of ABNORMALITIES

Dose Level mg/kg	Air Cell		Yolk	
	0 hrs	96 hrs	0 hrs	96 hrs
2.000	-	0.458	-	-
5.000	0.052	0.111	0.522	0.259
10.000	-	0.472	-	-
25.000	0.019	0.001	0.003	3.814
50.000	0.264	1.189	0.139	0.647
100.000	0.066	0.357	0.862	1.519
125.000	0.000	0.082	0.147	0.280
All Doses (DF)	2.633(5)	7.164(7)	2.711(5)	7.953(5)

TABLE 8

SODIUM TRIPOLYPHOSPHATE
PROBIT ANALYSES - ABNORMALITIES

Air Cell		Yolk	
0 hrs	96 hrs	0 hrs	96 hrs
NS	NS	NS	NS

TABLE 9

SODIUM TRIPOLYPHOSPHATE
CHI-SQUARE ANALYSES of HLSV ABNORMALITIES

Dose Level mg/kg	Air Cell		Yolk	
	0 hrs	96 hrs	0 hrs	96 hrs
2.000	-	0.458	-	-
5.000	0.052	0.111	0.001	0.022
10.000	-	0.472	-	-
25.000	0.019	0.470	0.643	0.069
50.000	0.264	0.451	0.068	0.259
100.000	0.066	0.357	0.017	0.061
125.000	0.000	0.082	0.261	0.000
All Doses (DF)	2.633(5)	5.076(7)	4.263(5)	2.668(5)

TABLE 10
SODIUM TRIPOLYPHOSPHATE in WATER

TERATOGENIC FINDINGS

TREATMENT	TOTAL NO. EXAMINED	TOTAL NO. ABNORMAL	SPECIFIC FINDINGS	
			NO.	D E S C R I P T I O N
Untreated Control	387	4	1	ataxia
			1	exencephaly; abnormal shortening-maxilla
			1	abnormal shortening-maxilla; dysgnathia-beak; celosomia-abdomen
			1	abnormal shortening-maxilla
Drilled Control - 0 hrs	125	1	1	hemorrhage
Drilled Control - 96 hrs	118	1	1	cachexia
Pierced Control - 0 hrs	122	2	1	dwarfism
			1	celosomia-abdomen
Air Cell - 0 hrs 125.0 mg/kg	166	2	1	anophthalmia
			1	celosomia-abdomen
100.0	123	2	1	anophthalmia; exencephaly; agenesis-maxilla; celosomia-abdomen
			1	celosomia-abdomen
50.0	124	1	1	celosomia-abdomen
5.0	127	2	1	celosomia-abdomen
			1	fusion failure-abdomen

TABLE 10
SODIUM TRIPOLYPHOSPHATE in WATER
TERATOGENIC FINDINGS

TREATMENT		TOTAL NO. EXAMINED	TOTAL NO. ABNORMAL	SPECIFIC FINDINGS NO.	DESCRIPTION
Air Cell - 0 hrs	0.0 mg/kg	165	1	1	anophthalmia
" " - 96 hrs	100.0	178	1	1	dwarfism; celosomia-abdomen
	50.0	177	4	1	microphthalmia; dysgnathia-beak; abnormal curvature-toe
				1	celosomia-abdomen
				1	agenesis-down
				1	dwarfism; celosomia-abdomen
	25.0	216	2	1	dwarfism
				1	abnormal curvature-toe
	5.0	119	1	1	anophthalmia; exencephaly; abnormal shortening-maxi
	0.0	209	1	1	celosomia-abdomen
Yolk - 0 hrs	125.0	122	2	1	cachexia
				1	abnormal shortening - maxilla
	100.0	126	1	1	hemorrhage-umbilical cord
	50.0	121	2	1	dysgnathia-beak
				1	anophthalmia; dysgnathia-beak; fusion failure-abdomen
	25.0	122	3	1	dysgnathia-beak
				1	exencephaly
				1	celosomia-abdomen

TABLE 10
SODIUM TRIPOLYPHOSPHATE in WATER
TERATOGENIC FINDINGS

TREATMENT		TOTAL NO. EXAMINED	TOTAL NO. ABNORMAL	SPECIFIC FINDINGS NO.	DESCRIPTION
olk - 0 hrs	5.0 mg/kg	158	2	1	celosomia-abdomen
				1	anophthalmia; exencephaly; abnormal shortening-maxilla; celosomia-abdomen
	0.0	163	5	1	hypopigmentation-down
				1	cachexia
				2	agenesis-down
				1	anophthalmia; exencephaly; dysgnathia-beak
olk - 96 hrs	125.0	154	3	1	abnormal shortening-maxilla
				1	exencephaly
				1	hypopigmentation-down
	100.0	119	4	1	hypopigmentation-down
				1	dwarfism; hypopigmentation-down
				1	dwarfism; fusion failure-abdomen
				1	exencephaly
	50.0	118	3	1	hypopigmentation-down
				1	dwarfism
				1	exencephaly
	25.0	117	6	1	hypopigmentation-down
				2	dwarfism
				1	abnormal curvature-toe
				1	cachexia
				1	anophthalmia

STUDIES on the TOXICITY and TERATOGENICITY of SODIUM TRIPOLYPHOSPHATE in AVIAN EMBRYOS

SUMMARY and CONCLUSIONS

Sodium tripolyphosphate produced a significant increase in embryo mortality in comparison with solvent-injected controls in each of the four test protocols. This compound was, therefore, found to be embryotoxic under the conditions of the studies.

The occurrence of abnormalities among the sodium tripolyphosphate-injected eggs was relatively low, and statistical analyses of these data failed to demonstrate a significant difference due to administration of this compound. It is, therefore, concluded that sodium tripolyphosphate was not teratogenic in the avian embryo under the conditions employed in these studies.

A 31

SODIUM TRIPOLYPHOSPHATE

TOXICITY and TERATOGENICITY STUDIES in Avian Embryos

FDA Contract #72-345

B.L. Reid

Department of Poultry Science
University of Arizona, Tucson, Arizona 85721